

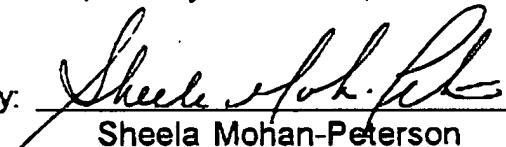
In view of the above, Applicants respectfully request reconsideration of the Restriction Requirement. Applicants will address the issue of inventorship for the elected claims and amend inventorship appropriately if the elected restriction is made final.

Applicants reserve the right to file subsequent applications claiming the non-elected subject matter and do not waive any of their rights or abandon any non-elected subject matter. Since Applicants have fully and completely responded to the Restriction Requirement and have made the required election, this application is now in order for early action.

If the Examiner believes that a telephonic conference would aid the prosecution of this case in any way, please call the undersigned.

Respectfully submitted,

Date: October 19, 2001

By: 
Sheela Mohan-Peterson
Attorney for Applicants
Reg. No. 41,201

DNAX Research Institute
901 California Avenue
Palo Alto, California, 94304-1104
Tel. No. (650) 496-6400
Fax No.: (650) 496-1200

Encl.:

Clark, Immunol. Today 21, 397 (2000).

Fig. 10-15 in Abbas, et al. (2000) Cellular and Molecular Immunology, 4th ed.,
W.B. Saunders Co., N.Y., p 230.

FOURTH EDITION

CELLULAR AND MOLECULAR IMMUNOLOGY

Abul K. Abbas, MBBS

Professor and Chair
Department of Pathology
University of California-San Francisco School of Medicine
San Francisco, California

Andrew H. Lichtman, MD, PhD

Associate Professor of Pathology
Harvard Medical School
Brigham and Women's Hospital
Boston, Massachusetts

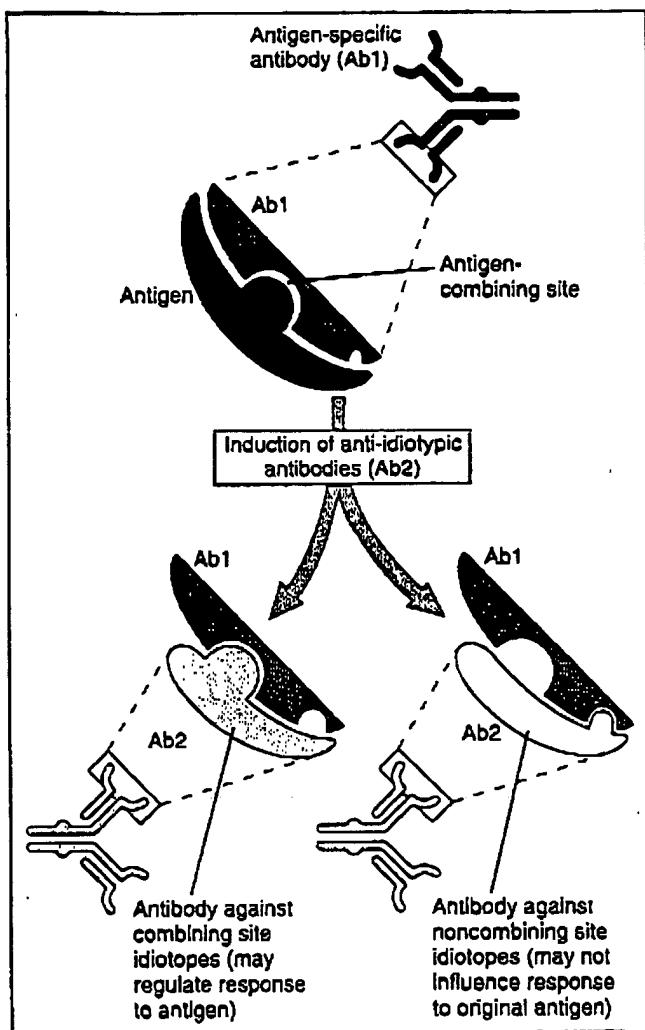
Jordan S. Pober, MD, PhD

Professor of Pathology, Immunobiology, and Dermatology
Yale University School of Medicine
New Haven, Connecticut

W.B. SAUNDERS COMPANY

A Harcourt Health Sciences Company
Philadelphia London New York St. Louis Sydney Toronto

230 Section III MATURATION, ACTIVATION, AND REGULATION OF LYMPHOCYTES

**Figure 10-15** Idiotypes and anti-idiotypic antibodies.

The combining site of an antibody (Ab1) specific for an antigen has a unique shape that is complementary to the antigen. Anti-idiotypic antibodies (Ab2) may recognize the combining site of Ab1, in which case they may influence responses to the antigen, or they may recognize unique idiotypic determinants of Ab1 that are not part of the combining site.

SUMMARY

- Immunologic tolerance is unresponsiveness to an antigen induced by the exposure of specific lymphocytes to that antigen. Tolerance to self antigens is a fundamental property of the normal immune system, and the failure of self-tolerance leads to autoimmune diseases. Antigens may be administered in ways that induce tolerance rather than immunity, and this may be exploited for the prevention and treatment of transplant rejection and autoimmune and allergic diseases.
- Central tolerance is induced in the generative lymphoid organs (thymus and bone marrow) when immature lymphocytes encounter self antigens present in these organs. Peripheral tolerance occurs when mature lymphocytes recognize self

antigens in peripheral tissues under particular conditions.

- The principal mechanisms of tolerance are deletion (apoptotic cell death), anergy (functional inactivation), and suppression by regulatory T cells. Some self antigens may be ignored by the immune system, and they elicit no detectable reaction.
- In T lymphocytes, central tolerance (negative selection) occurs when double-positive thymocytes with high-affinity receptors for self antigens recognize these antigens in the thymus. Several mechanisms account for peripheral tolerance in mature T cells. In CD4⁺ T cells, anergy is induced by antigen recognition without adequate costimulation and by recognition of altered forms of the native antigen. Repeated stimulation of T cells by persistent antigens results in activation-induced cell death. Some tolerogenic antigens activate suppressor T cells, which inhibit immune responses mainly by producing immunosuppressive cytokines.
- In B lymphocytes, central tolerance is induced when immature B cells recognize multivalent self antigens in the bone marrow. The usual result is apoptotic death of the B cells or the acquisition of a new specificity, called receptor editing. Mature B cells that recognize self antigens in the periphery in the absence of T cell help may be rendered anergic or are excluded from lymphoid follicles and cannot be activated by antigen.
- Immune responses to foreign antigens decline with time after immunization. This is mainly because of apoptosis of activated lymphocytes that are deprived of survival stimuli as the antigen is eliminated and innate immunity wanes. Various active mechanisms of lymphocyte inhibition may also function to terminate immune responses.

Selected Readings

Goodnow CC, JG Cyster, SB Hartley, SE Bell, MP Cooke, JI Healy, S Akkaraju, JC Rathmell, SL Pogue, and KP Shokat. Self-tolerance checkpoints in B lymphocyte development. *Advances in Immunology* 59:279-368, 1995.

Healy JI, and CC Goodnow. Positive versus negative signaling by lymphocyte antigen receptors. *Annual Review of Immunology* 16:645-670, 1998.

Klinman NR. The "clonal selection hypothesis" and current concepts of B cell tolerance. *Immunity* 5:189-195, 1996.

Lenardo M, FKM Chan, F Hornung, H McFarland, R Siegel, J Wang, and L Zheng. Mature T lymphocytic apoptosis—immune regulation in a dynamic and unpredictable antigenic environment. *Annual Review of Immunology* 17:221-253, 1999.

Matzinger P. Tolerance, danger, and the extended family. *Annual Review of Immunology* 12:991-1045, 1994.

Miller JFAP, and RA Flavell. T cell tolerance and autoimmunity.

48 Posey, J.A. et al. (1999) A pilot trial of GM-CSF and MDX-H210 in patients with erbB-2-positive advanced malignancies. *J. Immunother.* 22, 371-379

49 Valone, F.H. et al. (1995) Phase Ia/b trial of bispecific antibody MDX-210 in patients with advanced breast or ovarian cancer that overexpresses the proto-oncogene HER-2/neu. *J. Clin. Oncol.* 13, 2281-2292

50 Weiner, L.M. et al. (1995) Phase I trial of 2B1, a bispecific monoclonal antibody targeting c-erbB-2 and Fc_YRIII. *Cancer Res.* 55, 4586-4593

51 Pfister, D.G. et al. (1999) A phase I trial of the epidermal growth factor receptor-directed bispecific antibody MDX-447 in patients with solid tumors. *Proc. Am. Soc. Clin. Oncol.* 18, 67

52 De Gramont, A. et al. (2000) Adaptive immunotherapy in ovarian cancer with monocyte derived activated killer cells mixed with anti HER-2/neu x anti Fc_YRII bispecific antibody. *Proc. Am. Soc. Clin. Oncol.* 17, 95

53 Wu, J. et al. (1995) An activating immunoreceptor complex formed by NKG2D and DAP10. *Science* 265, 730-732

54 Takeuchi, O. et al. (1998) Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* 11, 443-451

55 Tomizuka, K. et al. (2000) Double trans-chromosomal mice: maintenance of two individual human chromosome fragments containing Ig heavy and κ loci and expression of fully human antibodies. *Proc. Natl. Acad. Sci. U. S. A.* 97, 722-727

Antibody humanization: a case of the 'Emperor's new clothes'?

Mike Clark

In the fable of the 'Emperor's New Clothes', a tailor tricks the Emperor into believing that he has a new and magical set of clothes, when in fact he does not. Most of the Emperor's subjects are equally taken in by the charade, too gullible, or too frightened to question what they are told. One small boy says what he actually sees and the trickery is eventually exposed. Without taking the analogy too far, I argue that antibody engineering has provided therapeutic antibodies with many new sets of clothes, and there are many competing claims as to which are better than the others. Not all these claims have a sound scientific basis and there is little evidence of rigorous scientific proof in support of some of these claims, particularly regarding immunogenicity of antibody variable regions.

It is 25 years since Kohler and Milstein published their work on the use of cell fusion for the production of monoclonal antibodies from immunized mice¹. The technique was rapidly and widely adopted and has provided an enormous repertoire of useful research reagents (see Little et al., this Issue). In turn, these reagents have formed a key element in the rapid progress of our understanding of biology. Although murine-derived monoclonal antibodies have been widely applied in clinical diagnostics, they have had limited success in human therapy (see Glennie and Johnson, this Issue, p. 403). Two major problems in the therapeutic

The antiglobulin response is perceived as a major problem in the clinical development of therapeutic antibodies. Successive technical developments such as chimeric, humanized and, now, fully human antibodies claim to offer improved solutions to this problem. Although there is clear evidence that chimeric antibodies are less immunogenic than murine monoclonal antibodies, little evidence exists to support claims for further improvements as a result of more elaborate humanization protocols.

use of murine monoclonal antibodies were identified early on. First, although murine antibodies had exquisite specificity for therapeutic targets, they did not always trigger the appropriate human effector systems of complement and Fc receptors². Second, even when murine antibodies were identified that did work *in vivo*, the patient's immune system normally cut short the therapeutic window, as murine antibodies were recognized by a human anti-murine antibody immune response (HAMA)^{3,4}. An obvious exception to these generalizations has been the success of the mouse monoclonal antibody, ortho-clone OKT3, used in prevention of organ graft rejection and which, in 1986, was the first monoclonal antibody to be approved by the FDA for clinical use (see Glennie and Johnson, this issue).

However, in parallel with the advances in the production of monoclonal antibodies from hybridomas, other major technological advances were happening in recombinant DNA technology. More was being understood about how genes for immunoglobulins were organized and expressed by B cells and about how germline immunoglobulin genes were rearranged and mutated, to form the repertoire of functional immunoglobulin genes^{5,6}. Inevitably, the techniques of monoclonal antibody production and recombinant DNA technology were combined to try to resolve the problems that had arisen in the application of murine antibodies to

REVIEW

IMMUNOLOGY TODAY

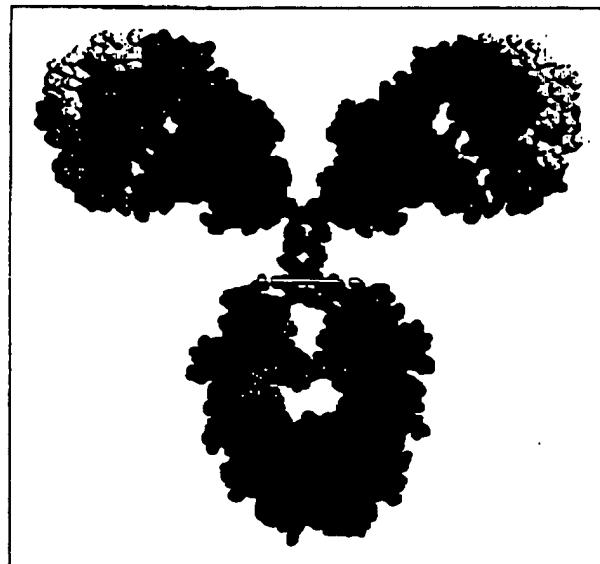


Fig. 1. Model of an IgG molecule. A spacefilling model of an IgG molecule coloured according to the structural features. Constant regions are in blue, carbohydrate in purple, variable region frameworks are in red and complementarity determining regions in yellow.

human therapy¹⁻². Thus, the field of antibody engineering was initiated, and today many of the products currently in clinical development by the biotechnology industry are recombinant monoclonal antibodies, or their derivatives (Glennie and Johnson, this issue, p. 403). It seems clear that recombinant DNA technology, as applied to antibody constant (C) regions, has provided solutions to the problems of antibody functions *in vivo*^{10,11}. But was this the right way to go for antibody variable (V) regions, or is it a case of the 'Emperor's new clothes'? Did enthusiasm to find ever more elaborate solutions to the HAMA response blind scientists to a more obvious shortcoming in the whole approach – namely human anti-human antibody

responses (HAHA). In particular to the idiotype¹²? In addition, is an anti-idiotype response only a function of the V-region sequence of the antibody or is immunogenicity more likely to depend on other factors^{12,13}?

IgG is the preferred class for therapeutic antibodies for several practical reasons¹¹. IgG antibodies are very stable, and easily purified and stored. *In vivo* they have a long biological half-life that is not just a function of their size but is also a result of their interaction with the so-called Brambell receptor (or FcRn)^{14,15}. This receptor seems to protect IgG from catabolism within cells and recycles it back to the plasma. In addition, IgG has subclasses that are able to interact with and trigger a whole range of humoral and cellular effector mechanisms. These mechanisms are initiated through immune complex formation, activation of complement, and through binding to cellular Fc receptors and complement receptors¹¹.

Figure 1 shows the basic structural features of a human IgG1. Figure 2 is a cartoon that illustrates how recombinant DNA technology can be used to transform a murine antibody into a human antibody for therapeutic applications. The immunoglobulin molecule is made up of a set of globular domains. Thus, antibody engineering can be applied in a modular fashion to generate a chimeric antibody with murine V-regions and human C-regions. Humanized antibodies can be generated where the antigen-binding complementarity determining regions (CDRs) are murine, while the rest of the antibody, including the V-region framework regions (FRs), is human.

Chimeric antibodies with human effector functions

The overall functions of an antibody are related to the constant regions, which determine the class and subclass. Clearly, human effector functions have evolved alongside human immunoglobulins. Very early during the development of rodent monoclonal antibodies for human therapy, it became clear that most monoclonal antibodies were ineffective in situations where cell destruction was a desired outcome (e.g., in cancer therapy). In some situations where several similar antibodies were used to target the same antigen, cell depletion was dependent on the antibody class and subclass¹⁶. Several studies have shown that human IgG1 is the preferred choice for chimeric antibodies in situations where activation of effector functions is the desired outcome⁷⁻¹¹.

There might be other occasions in therapy when the effector activity of IgG1 is not required. In this situation it is possible to make use of the human subclasses IgG2 and IgG4, which lack some of the effector functions¹¹. However, all four human IgG subclasses mediate at least some biological functions. Antibody engineering has again been applied to this problem and antibodies with altered Fc regions, and thus altered activity, have been produced. For example, removal of the conserved N-linked glycosylation site

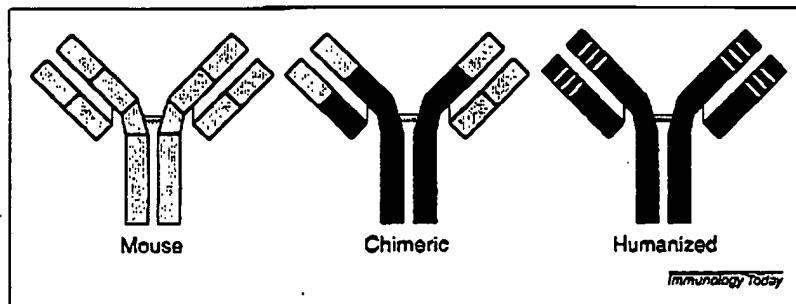


Fig. 2. Humanization of an IgG molecule. The blue represents mouse sequences and the red human sequences. In a chimeric antibody, the mouse heavy- and light-chain variable region (V-region) sequences are joined onto human heavy-chain and light-chain constant regions (C-regions). In humanized antibodies, the mouse complementarity determining regions sequences (three from the heavy-chain V-region and three from the light-chain V-region) are grafted onto human V-region framework regions and expressed with human C regions.

REVIEW
IMMUNOLOGY TODAY

on the constant region of a CD3 antibody reduces the unwanted biological side effects associated with Fc receptor binding, while retaining efficacy in blocking T-cell function¹⁷. An alternative strategy has recently been described whereby potent blocking antibodies can be generated by making antibodies with sequences derived from a combination of IgG1, IgG2 and IgG4 (Ref. 18).

Humanization to avoid the immune response

Immunogenicity of therapeutic antibodies is a significant problem and severely limits their widespread and repeated application to treat many diseases^{12,13}. Factors likely to be important in immunogenicity are listed in Box 1. Many believe that the immunogenicity of an immunoglobulin is mainly a function of its 'foreignness' to the human patient (top three items in Box 1). Most strategies for engineering antibodies to reduce immunogenicity are based upon the immunological concept that we are tolerant of all self-proteins and respond only to foreign proteins. Most immunologists would, I hope, recognize that this basic idea is flawed, particularly for immunoglobulins. By definition, every clone of B cells with a unique specificity has a unique V-region sequence: it possesses a unique 'idiotype'. New clones of B cells are generated every day throughout life by the processes of somatic gene recombination and somatic mutation¹⁸. It seems implausible that tolerance to each B-cell clone is generated as soon as every new sequence emerges. Indeed, an equally familiar immunological concept is the opposite extreme: that every clone of B cells provokes an anti-idiotype response, which in turn provokes another anti-idiotype, thus forming an antibody network that regulates immune responses¹⁹. The true situation is probably somewhere between these two extremes.

Four basic antibody engineering strategies have been adopted to tackle the immunogenicity of therapeutic antibodies. In chimeric antibodies, the murine constant regions are replaced with human constant regions, on the basis that the constant region contributes a significant component to the immunogenicity^{7,8,12}. As a further step on from chimeric antibodies, V-region humanization, or reshaping, involves changing some of the FR residues of the V-regions to sequences considered more 'human', while retaining the CDR sequences necessary for antigen binding (see Fig. 2; Refs 9,20–22). Two additional strategies provide what some call 'fully human V-regions': human antibody V-regions can be selected from phage libraries by affinity selection on antigen^{23,24}; transgenic mice have been constructed which have had their own immunoglobulin genes replaced with human immunoglobulin genes so that they produce human antibodies upon immunization^{25,26}.

Immunogenicity versus antigenicity of therapeutic antibodies

Before discussing just how sensible it is to promulgate the idea that 'V-region humanization' or 'fully human V-regions' are the way ahead, we need to consider the immunological difference between the concepts of 'antigenicity' and 'immunogenicity'. Antigenicity is defined in terms of the ability of a molecule to be recognized by

Box 1. Factors likely to influence immunogenicity of therapeutic antibodies

Murine constant regions

V-region sequences

Human Ig allotypes

Unusual glycosylation

Method of administration

Frequency of administration

Dosage of antibody

Patients' disease status

Patients' immune status

Patients' MHC haplotype

Specificity of antibody

Cell-surface or soluble antigen?

Formation of immune complexes with antigen

Complement activation by antibody

Fc receptor binding by antibody

Inflammation and cytokine release

All of these factors are likely to have a bearing on the immunogenicity of a therapeutic antibody. Many of them might be an inevitable consequence of the treatment and of the desired mode of action of the antibody. Only the first three on the list are altered by humanization and the fourth is likely to depend on the expression system used for antibody production.

preformed antibodies. A given antigen might be recognized by many different antibody specificities and these define the antigenic 'epitopes'. Immunogenicity refers to the ability of an antigen to provoke an immune response. It is possible for an antigen to be highly immunogenic, weakly immunogenic, nonimmunogenic or tolerogenic²⁷. For example, high doses of disaggregated human IgG given intravenously to mice can be tolerogenic, but the same antigen given in aggregated form subcutaneously can be highly immunogenic. It is the immunogenicity that is important for therapy, not the antigenicity^{12,13,27}.

For protein antigens such as immunoglobulins the immune response is usually T-cell dependent and there are many factors that might have a significant influence on the immunogenicity, including the need for appropriate antigen processing and presentation, and 'secondary signals'. How might recombinant antibodies influence these requirements? We should not lose sight of the fact that antibodies have evolved to play a key role in the immune response to infection and thus have features that often enhance immunogenicity of antigens bound up in immune complexes with the antibodies. It seems unlikely that at the same time as enhancing immunogenicity of the bound antigen the antibody is completely inert with regard to its own immunogenicity (particularly for its idiotype). Binding to Fc receptors²⁸, and the activation of the complement cascade^{29,30}, are two obvious ways that antibodies can enhance immune responses. In addition, antibodies that directly bind to cell surfaces might be internalized and processed for presentation²⁷, whereas antibodies that

REVIEW

IMMUNOLOGY TODAY

Table 1. Percentage homology of humanized and chimeric antibody V-regions^{a,b}

Antibody comparisons	FR	CDR	Complete V-region
Humanized V_H (i.e. complementarity determining regions grafted) versus human myeloma V_H			
Campath-1H versus NEW	98%	9%	74%
Anti-Tac versus EU	86%	28%	72%
OKT3 versus KOL	85%	25%	69%
Chimeric V_H (i.e., murine) versus human germline V_H			
Campath-1G	78%	41%	68%
Anti-Tac	77%	69%	75%
OKT3	77%	56%	72%
Human myeloma V_H versus human germline V_H			
NEW	87%	50%	78%
EU	95%	77%	91%
KOL	91%	81%	88%

^aAbbreviations: CDR, complementarity determining region; FR, framework region; V region, variable region.

^bThe percentage amino acid homology for several antibody heavy-chain V-regions is shown (from Ref. 34).

¹Humanized antibodies (Campath-1H, anti-Tac, OKT3) shown compared with the human myeloma protein sequences used as acceptor sequences for the humanization (NEW, EU, KOL).

²Murine sequences compared to the closest matching human germline immunoglobulin sequences found in the databases, i.e. the homology for chimeric versions of the antibodies.

³Comparison of the three human myeloma sequences with human germline immunoglobulin sequences.

cause cell killing and destruction might give rise to what are sometimes referred to as 'danger' signals³¹. In support of these ideas, it has been reported that the presence of the murine Fc region might make antibodies more immunogenic and also that Fab fragments are much less immunogenic than are F(ab')₂ or whole antibodies³². Additionally, antibodies that have been engineered so as not to activate effector functions are less immunogenic³³. Unfortunately, those properties of antibodies that result in enhanced antigen presentation are likely to be related to the desired properties of the antibodies that have been deliberately chosen for their therapeutic effect^{34,35,36}. It might thus prove very difficult to generate tolerance even to humanized or fully human antibodies that cause such effects *in vivo*.

There is some hope from strategies whereby tolerance can be induced to a non-cell-binding, non-inflammatory antibody that has a slightly modified but otherwise similar idiotype to a cell-binding therapeutic antibody^{33,37,38}. However, the therapeutic antibody will still have a unique idiotype even compared with the deliberately engineered tolerizing antibody and so at best, this strategy might reduce or delay the probability of an anti-idiotype response, but is unlikely to eliminate it in all therapeutic circumstances. Other strategies involve identifying and engineering out the major peptide sequences that would activate helper T cells when presented on major histocompatibility complex (MHC) Class II molecules. The problem

here is that there are so many different alleles of MHC Class II in the human population that it would only be possible to do this for a limited number of the more common epitopes.

Humanized versus chimeric antibodies

So are 'humanized' antibodies, or even so called 'fully human' antibodies, likely to be better than 'chimeric' antibodies for human therapy? This has implications at several levels. Humanization of the V-regions might be considered to try to reduce potential problems of immunogenicity^{3,20}. However, during the humanization process, the antibody affinity is frequently reduced³. This reduction in affinity might be minimized by careful selection of human FRs that are homologous to the starting antibody^{31,22,34} or by reintroduction of important murine FR residues back into the engineered antibody^{3,21,34}. This strategy could result in a humanized antibody that is itself very homologous to a chimeric form of the same antibody. This can be easily seen in Table 1, where the murine V-regions of the antibodies anti-Tac and OKT3 have a similar overall percentage homology to a closest match human germline gene (75% and 72%) compared

with the homology of the humanized antibodies and the acceptor myeloma protein sequences (72% and 69%). Indeed, I am almost tempted to argue that humanization of these two antibodies has reduced their homology. The humanization methods used have maximized the homology for FRs at the expense of the homology for CDRs, however, the overall percentage homologies for chimeric and humanized versions of these antibodies are similar. Furthermore, the homology of chimeric antibodies compared with the human germline is only slightly less than some human myeloma protein sequences compared with the germline. This is presumably a consequence of somatic mutations in the myeloma protein sequences.

The similar homology of humanized and chimeric forms of an antibody as shown in Table 1 arises because V-regions are often much more homologous between species than are constant (C) regions. Although this seems counter-intuitive, the explanation is simple: there are many different human V-regions³⁵⁻³⁷ to compare with any one chosen murine V-region, but there are only four human IgG C-regions. However, there is little scientific evidence to support a substantial reduction in immunogenicity when the penalty is an antibody of perhaps lower affinity^{3,21,22} and one where the commercial viability is reduced further by the need to pay out numerous royalties for licences that cover the antibody engineering process. Humanization might increase the production costs of a therapeutic

REVIEW

IMMUNOLOGY TODAY

antibody substantially. If these cost increases are passed on to patients, some healthcare providers might consider the treatment too expensive to be used routinely. Then, ironically, although the percentage of patients showing antiglobulin responses might be reduced, the total numbers of patients treated will also be reduced. My recommendation is that, at present, it is well worth carrying out a cost-benefit analysis for humanization on every new antibody. If humanization means that the costs outweigh the benefit, then it might well be better to produce and use a chimeric form of the antibody for therapy.

Testing the efficacy of V-region humanization

What would constitute a 'proper scientific test' of humanization of V-regions as a means of reducing the immunogenicity? At a minimum, this would probably require the generation of humanized and chimeric forms of several different antibodies with different idiotypes. These pairs of antibodies would then have to be used in similar clinical studies where the number of patients is high enough to establish reliable immunogenicity frequencies. A significant result would indicate that all the humanized antibodies are less immunogenic than their chimeric counterparts. Such an experiment is unlikely to be contemplated on commercial or on ethical grounds. Furthermore, there is already considerable evidence for a wide variability in measured immunogenicity of chimeric and humanized antibodies to different antigens and used in different diseases^{12,13,18}. Thus, it can already be concluded that other factors listed in Box 1 might be more important than the V-region sequences.

So what can be done? We can return to animal experiments. It is possible to make anti-idiotype antibodies to rodent antibodies by immunizing the same strains from which the original monoclonal antibodies were derived¹⁹. Thus, rodents are clearly not tolerant of antibodies from the same species and strain. However, deliberately generating an anti-idiotype is often not easy, and usually requires the use of adjuvant or coupling to 'carrier' proteins such as keyhole limpet haemocyanin (KLH)²⁰. By contrast, it is also possible to generate tolerance to foreign proteins in rodents, including human immunoglobulins, as mentioned earlier^{13,17,21}. Animal models suggest that the nature of the target antigen is more important than the sequence of the antibody V-regions, with cell-binding antibodies being the most difficult with which to achieve tolerance²¹.

Alternative strategies for producing 'human' antibodies

Other strategies for the production of 'fully human' antibodies include phage libraries^{22,23} or transgenic mice^{25,26}, both of which make use of repertoires of human V-regions. A third technique consists of reconstituting severe combined immunodeficient (SCID) mice with human peripheral blood lymphocytes, immunizing these animals with antigen, followed by rescuing the immune B cells by fusion with myeloma cells²⁴. However, antibodies generated using these methods still have unique idiotypes, and in the case of antibodies from transgenic mice (and of course antibodies from reconstituted

SCID mice), the somatic mutations are in FRs as well as in the CDRs (Ref. 26). Some like to claim that chimeric antibodies are overall about 75% human in sequence (% homology of whole IgG), whereas humanized antibodies are 95% human and antibodies from transgenic mice or phage display are 100% human. These figures can only be derived if murine and human antibodies are thought of as being totally different (Fig. 2). In fact, as illustrated in Table 1, there is considerable sequence homology between mouse and human immunoglobulin sequences, as would be obvious to anyone who looks at the sequence databases^{22,23}. The Kabat database shows clearly that many V-region sequences, particularly for FRs, are conserved between species¹¹. This, combined with the large repertoire and diversity of sequences in each species, makes it likely that most rodent sequences have a homologous human counterpart (see Table 1; Refs 22,34-37,41). Equally, somatic mutations in antibodies from transgenic and reconstituted mice, particularly during affinity maturation, means that they are no longer 100% identical to inherited human germline genes, as is the case with human myeloma proteins (see Table 1; Ref. 34). The other problem with use of human antibodies generated by these methods is that it might still be necessary to engineer them as chimeric constructs to provide them with a suitable human C-region for therapy, particularly where novel C-regions are contemplated^{11,17,18}. Again, this has cost implications as it might then require royalty licence payments to cover both technologies.

Concluding remarks

For some therapeutic antibodies it seems likely that the problems of immunogenicity are likely to remain whatever the strategies chosen for their production. However, the anti-idiotype response is generally only a problem where repeated treatments are required for chronic and relapsing diseases such as in therapy of autoimmune disease^{12,13}. Indeed for many therapeutic applications in acute disease situations, the possibility of an anti-idiotype response is not likely to have a major impact on their efficacy in the vast majority of patients^{13,21}.

I would not want to imply that there is an absolute correlation between the fable of the Emperor's New Clothes and antibody engineering, but I think the scientific question of how the immunogenicity of an antibody V-region relates to its sequence must be addressed. It is unlikely, I argue, to be a simple matter of percentage homology. It is interesting to reflect on a comment written by Alan Munro to accompany the publication of the first descriptions of recombinant 'chimeric' antibodies⁴². Although he used the term 'chimaeric' where today we would refer to 'humanized', with some foresight, he wrote, 'by using the techniques of genetic engineering, it might be possible to obtain antibodies in which the antigen-binding site is defined by sequences from a rodent monoclonal antibody of the right specificity whereas the rest of the molecule is as "human" in structure as possible... Possibly the human immune system will not recognize such chimaeric molecules as foreign, but there are good reasons to think that they will'. Yet, what was obvious to Munro in 1984 seems to have been forgotten or is ignored by many today.

REVIEW

IMMUNOLOGY TODAY

I thank my colleagues over the years for many stimulating discussions on antibody therapy and immunological tolerance. More importantly, I acknowledge the contributions of the undergraduates to whom I have taught immunology. Their probing questions bring home the shortcomings that still remain in our understanding of immunological problems.

Mike Clark (mrc7@cam.ac.uk) is in the Immunology Division, Dept of Pathology, Cambridge University, Tennis Court Road, Cambridge, UK CB2 1QP.

References

- 1 Kohler, C. and Milstein, C. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256, 495-497
- 2 Clark, M. et al. (1984) Advantages of rat monoclonal antibodies. *Immunol. Today* 4, 100-101
- 3 Schraff, R.W. et al. (1985) Human anti-murine responses in patients receiving monoclonal antibody therapy. *Cancer Res.* 45, 879-885
- 4 Shawler, D.L. et al. (1985) Human immune responses to multiple injections of murine monoclonal immunoglobulins. *J. Immunol.* 135, 1530-1535
- 5 Tonegawa, S. (1983) Somatic generation of antibody diversity. *Nature* 302, 575-581
- 6 Alt, F.W. et al. (1992) VDJ recombination. *Immunol. Today* 13, 306-314
- 7 Boulianne, G.L. et al. (1984) Production of functional chimaeric mouse/human antibody. *Nature* 312, 643-646
- 8 Morrison, S.L. et al. (1984) Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc. Natl. Acad. Sci. U. S. A.* 81, 6851-6855
- 9 Riechmann, L. et al. (1988) Reshaping human antibodies for therapy. *Nature* 332, 323-327
- 10 Hale, G. et al. (1988) Remission induction in non-Hodgkin lymphoma with reshaped human monoclonal antibody CAMPATH-1H. *Lancet* 2, 1394-1399
- 11 Clark, M.R. (1997) IgG effector mechanisms. *Chem. Immunol.* 65, 88-110
- 12 Kuus-Reichel, K. et al. (1994) Will immunogenicity limit the use, efficacy, and future development of therapeutic monoclonal antibodies? *Clin. Diagn. Lab. Immunol.* 1, 365-372
- 13 Isaacs, J.D. (1990) The antiglobulin response to therapeutic antibodies. *Semin. Immunol.* 2, 449-456
- 14 Chetie, V. et al. (1996) Abnormally short serum half-lives of IgG in beta-2-microglobulin-deficient mice. *Eur. J. Immunol.* 26, 690-696
- 15 Junghans, R.P. and Anderson, C.L. (1996) The protection receptor for IgG catabolism is the beta2-microglobulin-containing neonatal intestinal transport receptor. *Proc. Natl. Acad. Sci. U. S. A.* 93, 5512-5516
- 16 Dyer, M.J.S. et al. (1989) Effects of CAMPATH-1 antibodies *in vivo* in patients with lymphoid malignancies: influence of antibody isotype. *Blood* 73, 1431-1439
- 17 Bolt, S. et al. (1993) The generation of a humanised, non-mitogenic CD3 monoclonal antibody which retains *in vitro* immunosuppressive properties. *Eur. J. Immunol.* 23, 403-411
- 18 Annour, K.L. et al. (1999) Recombinant human IgG molecules lacking Fc-gamma receptor I binding and monocyte triggering activities. *Eur. J. Immunol.* 29, 2613-2624
- 19 Macias, A. et al. (1999) Novel cross-reactive anti-idiotype antibodies with properties close to human intravenous immunoglobulin (IVIg). *Hybridoma* 18, 263-272
- 20 Jones, P.T. et al. (1986) Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature* 321, 522-525
- 21 Queen, C. et al. (1989) A humanized antibody that binds to the interleukin 2 receptor. *Proc. Natl. Acad. Sci. U. S. A.* 86, 10029-10033
- 22 Gorman, S.D. et al. (1991) Reshaping a therapeutic CD4 antibody. *Proc. Natl. Acad. Sci. U. S. A.* 88, 4181-4185
- 23 Winter, G. et al. (1994) Making antibodies by phage display technology. *Annu. Rev. Immunol.* 12, 433-435
- 24 Low, N.M. et al. (1996) Mimicking somatic hypermutation: affinity maturation of antibodies displayed on bacteriophage using a bacterial mutator strain. *J. Mol. Biol.* 260, 359-368
- 25 Bruggemann, M. et al. (1991) Human antibody expression in transgenic mice: expression from 100kb of the human IgH locus. *Eur. J. Immunol.* 21, 1323-1326
- 26 Mendez, M.J. et al. (1997) Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice. *Nat. Genet.* 15, 146-156
- 27 Benjamin, R.J. et al. (1986) Tolerance to rat monoclonal antibodies: implications for serotherapy. *J. Exp. Med.* 163, 1539-1552
- 28 van-Vugt, M.J. et al. (1999) The Fc-gammaRIIa (CD64) ligand binding chain triggers major histocompatibility complex class II antigen presentation independently of its associated FcR gamma-chain. *Blood* 94, 808-817
- 29 Fearon, D.T. (1998) Non-structural determinants of immunogenicity and the B-cell co-receptors, CD19, CD21 and CD22. *Adv. Exp. Med. Biol.* 452, 181-184
- 30 Lou, D. and Kohler, H. (1998) Enhanced molecular mimicry of CEA using photolabelled crosslinked C3d peptide. *Nat. Biotechnol.* 16, 458-462
- 31 Matzinger, P. (1994) Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* 12, 991-1045
- 32 Routledge, E.G. et al. (1995) The effect of glycosylation on the immunogenicity of a humanized therapeutic CD3 monoclonal antibody. *Transplantation* 60, 847-853
- 33 Gilliland, L.K. et al. (1999) Elimination of the immunogenicity of therapeutic antibodies. *J. Immunol.* 162, 3663-3671
- 34 Routledge, E.G. et al. (1993) Reshaping antibodies for therapy. In *Protein Engineering of Antibody Molecules for Prophylactic and Therapeutic Applications in Man* (Clark, M., ed.), pp. 13-44. Academic Titles
- 35 Cook, C.P. and Tomlinson, I.M. (1995) The human immunoglobulin VH repertoire. *Immunol. Today* 16, 237-242
- 36 Williams, S.C. et al. (1996) Sequence and evolution of the human germline V lambda repertoire. *J. Mol. Biol.* 264, 220-232
- 37 Tomlinson, I.M. et al. (1995) The structural repertoire of the human V kappa domain. *EMBO J.* 14, 4628-4638
- 38 Rebello, P.R. et al. (1999) Anti-globulin responses to rat and humanized CAMPATH-1 monoclonal antibody used to treat transplant rejection. *Transplantation* 68, 1417-1420
- 39 Cobbold, S.P. et al. (1990) A simple method for measuring patient anti-globulin responses against isotypic or idiotypic determinants. *Methods Enzymol.* 127, 19-24
- 40 Lubin, I.H. et al. (1994) Engraftment of human peripheral blood lymphocytes in normal strains of mice. *Blood* 83, 2368-2381
- 41 Johnson, G. and Wu, T.T. (2000) Kabat database and its applications: 30 years after the first variability plot. *Nucleic Acids Res.* 28, 214-218
- 42 Munro, A. (1984) Uses of chimaeric antibodies. *Nature* 312, 597

Students...

Did you know you are entitled to a 50% discount on a subscription to *Immunology Today*?

See the bound-in subscription card for more details.